In this study we have used a combination of molecular modelling and experimental techniques to examine the structure of the TRPM8 TM and pore helix regions including conformation of the selectivity filter. We present structural insight into the role of residues involved in intra- and inter-subunit interactions and their link with the channel activity, sensitivity to icilin, menthol, and cold, and impact on channel oligomerization.

1690-Pos Board B420

Cellular Regulation of Transient Receptor Potential Melastatin 3 (TRPM3) Channel Activity

Balazs I. Toth, Joris Vriens, Debapriya Ghosh, Thomas Voets.
KU Leuven, Leuven, Belgium.

TRPM3 is a less characterized member of the melastatin subfamily of transient receptor potential (TRP) ion channels activated by the neurosteroid pregnenolone sulphate (PS) and heat. Recently we have shown that TRPM3 is a thermosensitive nociceptor channel involved in the detection of noxious heat and mediating the nocifensive behaviour evoked by PS. In our current study, we aimed at discovering intrinsic cellular factors regulating TRPM3 activity. We applied a combined pharmacological and molecular biological approach and investigated the channel activity by electrophysiological measurements and functional imaging on HEK293T cells overexpressing a wild-type variant of mouse TRPM3. In cell attached and inside out configuration of patch clamp measurements, we found that the composition of the intracellular milieu dramatically influenced the channel activity. By further studying cellular signaling mechanisms potentially involved in the intracellular regulation of TRPM3 we tested the role of adenosine nucleotides, phosphatidylinositol 4,5-bisphosphate ($PtdIns(4,5)P_2$) and various kinases, as well. We found that presence of adenosine 5-trisphosphate (ATP) crucially influenced the TRPM3 activity. This could involve both direct effects and events related to ATP metabolism. Although we found $PtdIns(4,5)P_2$ to be able to interact functionally with TRPM3, our results indicated that protein kinases mediated phosphorylation could be a more prominent factor in the regulation of TRPM3 activity. In our current experiments we are identifying specific protein kinases involved in the cellular control of TRPM3. Our results suggest that both phospholipids and adenosine nucleotides can modulate TRPM3 channel functions with a central role of phosphorylation processes.

1691-Pos Board B421

Novel TRPM3 Agonist - Single Compound Opens Multiple Ion Permeation Pathways

Katharina Held, Annelies Janssens, Patrick Chaltin, Thomas Voets, **Joris Vriens**.
K.U.Leuven, Leuven, Belgium.

TRPM3 is a calcium-permeable nonselective cation channel in the melastatin (TRPM) subfamily of TRP channels. It represents a typical example of a polymodally gated TRP channel, in that it can be activated by chemical ligands such as nifedipine and the neurosteroid pregnenolone sulphate (PS), as well as by physical stimuli such as heat and membrane depolarization. Recently, we have found evidence for an alternative ion permeation pathway distinct from the central pore, which can be gated by combined application of PS and exogenous chemicals such as clotrimazole. This alternative ion permeation pathway is preserved following desensitization, blockade, mutagenesis and chemical modification of the central pore, and enables massive Na^+ influx at negative voltages. By screening a compound library, we identified CIM021600 as potent agonist of TRPM3. The compound exhibited a marked specificity for TRPM3 and induced $[Ca^{2+}]_I$ signals in somatosensory neurons. Intriguingly, single application of CIM021600 was able to activate both the central pore ion permeation pathway and the alternative ion permeation pathway. Since physiological functions of TRPM3 and the alternative ion permeation pathway of TRPM3 are still poorly defined, the identification of a potent and selective activator is expected to contribute to clarifying the role of TRPM3 in vivo.

1692-Pos Board B422

Regulation of the Ion Channel TRPM3 by Phosphoinositides

Doreen Badheka, Tibor Rohacs.
Rutgers New Jersey Medical School, Newark, NJ, USA.

TRPM3 belongs to the Melastatin family of Transient Receptor Potential (TRP) ion channels. It is a Ca^{2+} permeable outwardly rectifying nonselective cation channel. TRPM3 is expressed in sensory neurons, brain, pancreas, kidney and vascular smooth muscle. In sensory dorsal root ganglion (DRG) neurons it was shown to function as a sensor for noxious heat. $TRPM3^{-/-}$ mice have decreased ability to detect noxious heat. In DRG neurons as well as pancreatic beta cells, the neurosteroid pregnenolone sulfate has been shown to activate TRPM3.

Many members of the TRP family are regulated by phosphatidylinositol 4,5-bisphosphate ($PtdIns(4,5)P_2$). Thus we decided to test if TRPM3 is also regulated by $PtdIns(4,5)P_2$. $PtdIns(4,5)P_2$ is predominantly present at the cytoplasmic face of the plasma membrane and it is an important signaling molecule. In the current study we modulated the levels of $PtdIns(4,5)P_2$ in *Xenopus laevis* oocytes and HEK cells heterologously expressing TRPM3. In inside-out patches, TRPM3 currents ran down rapidly after excision. This current could be restored by the exogenous application of water soluble diC_8 $PtdIns(4,5)P_2$ and the naturally occurring arachidonyl-stearyl (AASt) $PtdIns(4,5)P_2$. Also, application of MgATP to excised inside-out patches reactivated the TRPM3 current. This effect of MgATP was inhibited by LY294002 at a concentration where it inhibits phosphatidylinositol 4 kinase (300 μM) but not at a concentration where it inhibits phosphatidylinositol 3 kinase (10 μM). This suggests that MgATP acted via replenishing $PtdIns(4,5)P_2$ and PtdIns(4)P. In addition, inducing the activity of $PtdIns(4,5)P_2$-5-phosphatases in whole-cell patch clamp experiments also decreased TRPM3 currents. Overall our data collected through excised inside-out and whole-cell patch clamp measurements suggest that TRPM3 requires $PtdIns(4,5)P_2$ as a cofactor.

1693-Pos Board B423

Inhibition by Reduction of PIP2 Accelerates Inactivation of Receptor-Operated TRPC6/7 Currents

Mori Masayuki[1], Kyohei Itsuki[2], Yuko Imai[2], Hideharu Hase[1], Yasushi Okamura[3], Ryuji Inoue[4].
[1]Kyoto University, Kyoto, Japan, [2]Kyushu University, Fukuoka, Japan, [3]Osaka University, Osaka, Japan, [4]Fukuoka University, Fukuoka, Japan.

TRPC3, C6 and C7, which comprise a subfamily of the mammalian diacylglycerol (DAG)-activated TRPC cation channels, play key roles in cardiovascular, gastrointestinal, renal and nervous system physiology. We recently reported that the depletion of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) dominantly inhibits these TRPC channels activity, irrespective of the presence of a DAG analogue. However, the functional significance of PI(4,5)P2 reduction/depletion during G(q) protein-coupled receptor-operated TRPC activation remains largely unknown. To address this question, we simultaneously measured TRPC current kinetics and PI(4,5)P2/DAG dynamics using Förster resonance energy transfer (FRET) at varying degrees of receptor stimulation. Our measurements demonstrated a clear correlation between the time-course of activation/inactivation in TRPC6/7 currents and the kinetics of PI(4,5)P2 reduction. In contrast, the time-course of DAG production is incompatible with TRPC6/7 current inactivation, indicating that reduction of PI(4,5)P2 is primarily responsible for this inhibition. After estimating the functional dissociation constant of PI(4,5)P2 binding to these DAG-sensitive TRPC channels, we used a model simulation to demonstrate the similarity of receptor-operated TRPC6/7 currents in both a HEK293 cell recombinant expression system and aortic smooth muscle cells (A7r5). Our findings demonstrate that G(q) protein-coupled receptor stimulation causes a reduction in PI(4,5)P2 levels that inhibits TRPC6/7 channels by accelerating their inactivation and perhaps limiting their maximum open probability.

1694-Pos Board B424

Regulation of TRPM8 Channel Activity in Prostate Cancer by Androgens

Swapna Asuthkar[1], Lusine Demirkhanyan[1], Xiaohui Sun[1], Pia Elustondo[2], Kiran Velpula[1], Evgeny Pavlov[2], **Eleonora Zakharian**[1].
[1]Cancer Biology & Pharmacology, University of Illinois, Peoria, IL, USA, [2]Physiology & Biophysics, Dalhousie University, Halifax, NS, Canada.

The TRPM8 protein is a Ca^{2+}-permeable ion channel and may therefore play significant role in Ca^{2+}-homeostasis. The role of TRPM8 in peripheral nervous system is well established as a cold and menthol receptor. Unlike the sensory neurons, expression and physiological designation of TRPM8 channels in the normal prostate function and in the development of prostate cancer are entirely unclear. Previous studies showed that androgens (testosterone and 5-alpha-dihydrotestosterone) induced the mRNA expression of TRPM8 in prostate cancer cells. However, the mechanism behind their synergistic regulation and the molecular staging of prostate cancer is not fully understood. In the present study, we first investigated the intracellular location of TRPM8 in a panel of 60 cases of prostate adenocarcinoma (grade 1-4) and 9 cases of normal prostate tissues. We identified that unlike prostate cancer cell lines, prostate tumor tissues show high levels of TRPM8 which was predominantly localized as the plasma membrane protein at the tumor periphery. We also showed the expression of TRPM8 protein and its association with an androgen-sensitive (LNCaP, RWPE-2) and an androgen-insensitive (PC-3) prostate cancer and epithelial cells. Further, using chromatin-immunoprecipitation (ChIP) and calcium-imaging experiments, we identified that TRPM8 Ca^{2+} channel induction and functional activation is